Poster Presentation

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A novel method to stabilize weak protein complexes for crystallographic studies

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Most protein interactions mediating critical steps in cellular pathways are transient and, hence, are difficult to capture using structural approaches. An example from Saccharomyces cerevisiae is the interaction between checkpoint effector Rad53 kinase and replication initiation Dbf4-Cdc7 kinase. Dbf4 is the regulatory and Cdc7 the catalytic subunit of this kinase, which functions in activating replication origins. When a replication fork is damaged, the checkpoint response activates Rad53, which binds transiently, yet specifically, to Dbf4 and this, in turn, inhibits the activity of Dbf4-Cdc7. The outcome of this interaction prevents activation of late origins during replication stress. Our laboratory has extensively characterized the Dbf4-Rad53 interaction, thereby providing an excellent system to probe new ways to stabilize a weak protein complex. The N-terminal forkhead associated (FHA) domain of Rad53 mediates the interaction with the BRCA-1 C-terminus (BRCT) domain of Dbf4. FHA and BRCT domains are modular domains, in which their N- and C-termini lie on the same face. Thus, we decided to stabilize the Dbf4-Rad53 complex using linkers of different lengths to join the two proteins. We generated four different Dbf4-linker-Rad53 fusions and characterized them biochemically, as well as structurally using small angle X-ray scattering. Only one of the fusions yielded crystals suitable for crystallographic analysis, and we solved the structure by molecular replacement. The four copies of the fusion in the asymmetric unit showed identical Dbf4-Rad53 interfaces that were in agreement with previous studies characterizing the Dbf4-Rad53 interaction. Importantly, the interaction in the crystal structure occurs inter- rather than intra-molecularly suggesting that the linker increases local protein concentration but does not impose complex formation. In a broader sense, our work reveals general trends that can be used to design linkers to capture weak protein complexes.

[1] *Matthews et al (2012) J. Biol. Chem.,* ***[2]*** *Matthews et al (2014) J. Biol. Chem.*

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